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The Effects of Suspended Sediment on the Aquatic Organisms Daphnia magna and Pimephales promelas

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THE EFFECTS OF SUSPENDED SEDIMENT ON THE AQUATIC ORGANISMS *Daphnia magna* AND *Pimephales promelas*

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ABSTRACT

Land use change results in soil migration into aquatic streams during storm events. This suspended sediment, even in the absence of adsorbed contaminants, may be a significant stressor to aquatic organisms. In some parts of the US, total suspended solids (TSS) concentrations surpass 100,000 mg/L during storm events. The limited data on effects of suspended sediment concentrations on freshwater fishes has mainly focused on salmonids or ecosystem level consequences such as habitat alteration. Few studies have quantified sublethal physiologic effects of suspended solids on water column organisms. This research determined the effects of suspended clay-sized particles on *Daphnia magna* and *Pimephales promelas* (Fathead minnow).

D. magna, exposed to natural and defined clay-sized suspensions, had 7-d LC_{50} values ranging from 5 mg/L to 75 mg/L with montmorillonite being more toxic than kaolinite. Natural clay-sized particles, which were composed of approximately 60% kaolinite, exhibited a 7-d LC_{50} value of 51 mg/L, which may indicate a connection between particle source and toxicity. Suspended clay-sized particles rapidly filled the intestinal tract of daphnids ultimately leading to starvation. When transferred to clean media gut clearance occurred within 30 minutes suggesting quick recovery between pulsed exposures.

Fathead minnows were exposed to concentrations ranging from 50 mg/L to 1,000 mg/L of natural and defined clay-sized particles . Whole-body sodium, gill Na^+/K^+-ATPase activity, and percent body moisture were chosen as endpoints representing the ionoregulatory abilities of the fish. Only montmorillonite produced a statistically

significant effect in the form of increased gill Na^+/K^+-ATPase activity and decreased whole-body sodium concentrations. This effect was seen in the first 12 hours of exposure and is thought to have led to mortality in some organisms exposed to 1,000 mg/L montmorillonite.

This study was an effort to not only investigate sublethal effects of suspended clay-sized particles on aquatic organisms, but also an effort to standardize toxicity testing by exploring relationships between standard and non-standard particle types. The results of such comparisons, in the form of varying LC_{50} values and differing effects on gill enzyme activity, show that achieving reproducible effects using dissimilar sediment sources is unlikely. However, results of this research underscored the need to treat suspended sediment as a water column contaminant.

DEDICATION

I dedicate this work to my father, Eddie Alan Capper, who instilled his love for science and drive for excellence in all his children. Although he passed away while this research was being conducted, his guidance over the years has proved invaluable not only in my academic endeavors but in all aspects of my life.

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LITERATURE REVIEW

Suspended Solids in the Natural Environment

Suspended solids are a vital component of aquatic ecosystems and are responsible for transporting nutrients, providing microbial habitat, and serving as a food supply for many organisms [1]. The term suspended solids can refer to numerous particle types including suspended organic matter, such as leaves and decaying organisms, and inorganic matter such as suspended sediment. Suspended sediment is perhaps one of the most versatile forms of suspended solids and plays an important role in ecological, chemical, and physical processes in aquatic systems. Excessive suspended sediment, however, can exert several types of stress on aquatic ecosystems. These stresses include benthic habitat alteration (scouring or deposition), changes in light attenuation and subsequent changes in primary productivity, and physiological effects on organisms ranging from growth and reproduction inhibition to mortality [2-4]. Suspended sediment can also transport hydrophobic contaminants downstream [5-7] and increase the costs of water treatment and purification. Yearly water quality reports from the U.S. Environmental Protection Agency indicate approximately 40% of assessed river miles are impaired by sediment stress in some form [8].

Effects from suspended sediment can vary in severity based on several factors. One of the main factors is grain size. This property is typically classified based on the longest dimension of the individual particles and the three main categories are sand (20 to 2000 μm in diameter), silt (2 to 20 μm in diameter), and clay (<2 μm in diameter). These classifications have been determined by the International Society of Soil Scientists (ISSS) [9] and are not unanimously followed. While there are several other grain size classification scales, the differences are mostly within 20 µm of each other. Based on the above definition of grain sizes, particles classified as sands will have a larger effect on scouring and deposition than particles classified as clays since the smaller clays will stay suspended longer. Conversely, clays will have more effect on light attenuation in an ecosystem and, hence, impair primary production more than sand particles. As a result of this, most research regarding the effects of suspended sediment on aquatic ecosystems has focused on clay particles, with a smaller percentage including silt-sized particles. These smaller particles (clay and silt) are more likely to directly affect aquatic organisms through impacts on the feeding of planktivorous fish and filter-feeding invertebrates.

Land Use Effects on Sediment Loading

Soil is often transported into aquatic ecosystems as a result of land use change. Land use change implies an alteration, usually anthropogenic, in the use of a given parcel of land. For example, the development of a soybean field into suburban housing represents a significant disturbance. These changes involve the removal of vegetative cover which increases stormwater runoff that carries significant amounts of eroded soil into surface waters [3,10-11]. Best Management Practices (BMPs) have been designed to reduce the amount of soil transported from a given site during construction. BMPs can vary from structural modifications, such as slotted inlet pipes, to cultural practices such as deliberate planting of winter crops and reduced tillage. Such methods have been documented to significantly improve water quality in receiving basins [13]. In South Carolina, prescribed BMP's for land development have been designed to reduce soil discharge by at least 80%. The philosophy has been that by trapping at least 80% of soil being transported from the areas under management, the impact of such disturbances on aquatic systems is diminished. However, the remaining eroded soil (up to 20%) that is exported with these practices can be harmful to aquatic ecosystems. This is particularly true given the scale of land developments that are so large that even if BMPs achieve greater than 80% soil trapping efficiency, tons of eroded soil will still migrate into receiving streams. The majority of these exported soil particles are clay (<2 µm) and silt (2-20 µm) can stay suspended for days to weeks in surface waters, depending on local catchment features.

The addition of this soil to streams and rivers can affect the ability of the systems to support a diverse community. A study detailing species richness in Lake Tanganyika, which lies along the borders of Tanzania, Sudan, Burundi, and Zambia, found that highly disturbed localities yielded on average 62% lower aquatic species richness than in corresponding undisturbed lake areas [13]. Higher overland water discharge, coupled with increased suspended sediment results in scoured streambeds and destabilized stream banks and channels. Under these conditions stream hydrographs change significantly indicating rapid increases in water flow and significantly higher peak flows than undisturbed areas (Figure 1). The difference in discharge patterns illustrated in Figure 1 demonstrates the rapid spike in discharge following storm events that occurs in modified watersheds as opposed to the relatively gentle rise and fall of discharge typified by undisturbed watersheds.

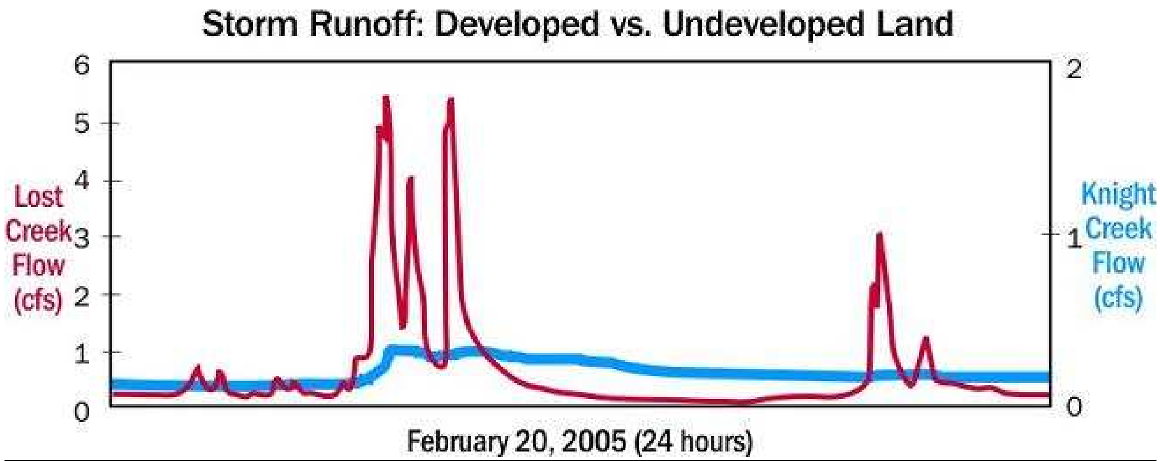

Figure 1: Comparison of hydrographs from disturbed and undisturbed land areas. Knight Creek drains an undisturbed reference watershed adjacent to a rapidly developing watershed drained by Lost Creek. On this date, The Lost Creek watershed was approximately 20% developed (disturbed).

Regulatory Review

Installing BMPs to reduce sediment export is often complicated by regulatory practices. Currently, there are no federal regulations with respect to suspended sediment in the natural environment. Decisions on regulation have been left largely to individual states, which have varying levels of standards. The USEPA conducted a study of published suspended sediment criteria in 2001 and found that only 32 of the 53 states and territories have numeric criteria for suspended sediment [8]. Thirteen of the states with no published numeric criteria reported narrative criteria. In May of 2006 the USEPA published guidelines to assist states in determining suspended solids criteria best suited for individual states. This general disarray with regards to regulation has made it difficult to set realistic goals in terms of sediment reduction resulting from BMP practices. Further complications exist in the form of differences between determination methods and units of measurement. Thirty of the 32 states with numeric guidelines use turbidity as their criteria with only two states using only suspended solids criteria. Turbidity is the easier of the two metrics to measure as it involves a small sample volume (<25 ml) and rapid test time, usually 30 to 60 seconds. Determining suspended solids is time intensive; it involves filtering and drying sample volumes that can exceed 500 ml. The relationship between turbidity and suspended solids is dependent on local conditions and direct conversion is not always possible. As turbidity is a measure of light scatter through a water sample, any component that will deflect or absorb light can affect the reading. Consequently, components of the sample other than sediment may alter the results. This can include constituents such as algal cells and humic acid components.

Furthermore, it has been established that smaller particles scatter light more uniformly than larger particles, and therefore turbidimeters are more sensitive to samples with a larger percentage of fine particles than larger ones [14-16]. These factors can confound comparisons between localities with different physical characteristics, such as the ones described above. The units of turbidity measurements are nephelometric turbidity units (NTU) and were established as the turbidity resulting from a suspension of 1 part per million silica. Formazine has more recently replaced silica due to its greater stability and is the current standard used for calibrating turbidimeters [17]. This compound is made from a mixture of hydrazine sulfate and hexamethylenetetramine. Measurements of suspended solids can be more indicative of suspended sediment only, as confounding elements such as humic acids can be effectively eliminated. Measuring suspended solids by weight also removes particle size influences from the measurement. Despite these complications most correlations between the two variables are reasonable but are also site specific and require a large sample population for an adequate relationship. It would thus be prudent to establish guidelines in one form or another and standardize all measurements to one variable.

Effects of Suspended Sediment on Fishes

The mode of toxicity of suspended sediment on fish is generally thought to involve impaired oxygen transfer at the gills, reduced ability to clear sediment from the gills, and diminished osmoregulation (Servizi and Martens 1991). Epithelial walls of gill filaments are necessarily thin in order to facilitate oxygen transfer into the bloodstream. Fine particles can adhere to the gill surface and cut off this gas exchange and cause the fish to suffer from anoxia, or lack of oxygen, and related symptoms [4]. It has been noted that the gills of morbid fish have been clogged with sediment [18] but this may be simple adhesion following mortality rather than a process that took place before death. However, Martens and Servizi [19] documented fine particles lodged between the lamellae of live salmon; particles fine enough to move between intracellular junctions eventually became lodged in the spleen. Thickening of the pillar system in the gill lamella during suspended sediment exposure may also impair oxygen transfer [20].

During the 1980s the condition of salmon populations in the Pacific Northwest resulted in a large body of research on the effects of suspended sediment exposure. Despite being adapted for cool, clear water, these fish were able to withstand high concentrations of suspended sediment for short durations (<14 d). Reported 96-h LC_{50} values for salmonids range from 1,200 mg/L for juveniles [21] to 164,500 mg/L for adults [22]. Although these concentrations would be high for streams salmonids usually inhabit, they are not unrealistic, as these same areas typically experience spikes in suspended solids concentrations from logging operations and development. These values are dependent on duration of exposure, as LC_{50} values fall rapidly as duration increases. 588-hr LC_{50} values reported for adult rainbow trout were only 4,250 mg/L [23]. Figure 2 shows a graphical representation of the general relationship between exposure concentration and duration for adult freshwater fish. Lethality thresholds for suspended sediments also vary widely with life stage. The difference between juveniles and adults can be seen in the references above, and the difference is even more striking for eggs and larvae. While there was 47% mortality for artic grayling fry exposed to 230 mg/L for 96 hr [24], rainbow trout eggs exposed to 120 mg/L for 384 hr showed morality rates of 60-70% [25].

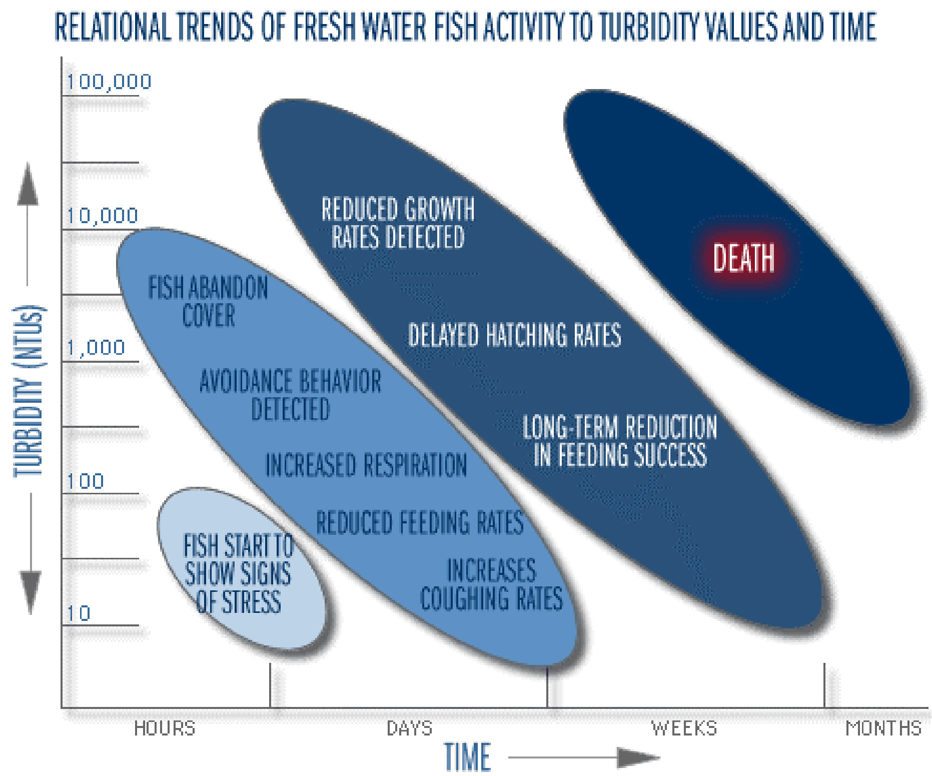

Figure 2: Effects of suspended sediment on fresh water fishes with respect to concentration and duration. From [33].

Suspended sediments may also cause sublethal effects in fishes. These effects can include symptoms typically associated with anoxia such as increases in hematocrit, red cell counts, and hemoglobin concentrations in the peripheral blood [4]. Other physiological effects have been noted such as suppression of the immune system and increases in plasma cortisol concentrations [22,26]. The occurrence of fin-rot has been reported to increase after exposure to suspended solids [22,27] and this trend is thought to be due to abrasion of epithelia that allow microbes to enter the fish. Increases in cortisol have also been found to lower immune system function [22,28] and this general stress response to suspended solids exposure could be the underlying cause of immunosuppression. Lake and Hinch [18] also reported that leukocrit, or percentage of white blood cells in the bloodstream, was significantly lowered following a 96-hr exposure to 1,000 mg/L crushed silica. Regardless of the mechanism, there is a significant body of literature to suggest that exposure to suspended sediment increases a fish's susceptibility to disease.

Further sublethal effects from suspended sediments arise from decreased light penetration through the water column. Aside from previously mentioned effects on primary productivity, decreases in water clarity can affect fish behavior. As with most predators, fish use sight as a primary sense in recognizing and capturing food. Although fish are known to use other detection methods such as chemosensory or lateral line reception, increases in suspended sediment would be expected to reduce feeding efficiency due to impaired vision. Rowe and Dean [29] demonstrated that feeding rates of several species of juvenile freshwater fish on *Daphnia magna* were significantly depressed after exposure to turbidity levels as low as 40 NTU's for 2 hr. Reduced feeding efficiency has also been shown in juvenile coho salmon during exposure to 60 NTU for five minutes [30]. The latter study also showed that the social hierarchy established within the study tank disintegrated at turbidity levels >30 NTU and decreased from six levels to two. This shows the strength of visual cues involved in behavior, as once turbidity was reduced to <20 NTU the original hierarchy was reasserted.

Turbidity can also affect reproductive behavior. Tricolor shiners (*Cyprinella trichroistia*) exposed to up to 600 mg/L suspended silt and clay for 5 days laid fewer eggs, exhibited delayed spawning activity and decreased spawning effort [31]. The authors estimated that similar conditions in the field would result in approximately 20% fewer eggs laid. This reduced spawning is most likely an effect caused from both stress and reduced visual cues used in mating. As stated previously, disruption of social hierarchy is another sublethal effect of suspended sediment that affects mating behavior. Dominant males will no longer defend their territory due to lack of visual cues from encroaching males [30]. This disruption makes it difficult for males to fertilize and guard their eggs. Not surprisingly, juvenile coho salmon have been shown to actively avoid clouds of suspended sediment and exhibit fright responses when forced into such clouds [32].

The effects of suspended sediment on warm-water, non-salmonid species have been less rigorously examined. The data that do exist suggests these species may be more sensitive to suspended sediment than salmonid species [33]. Few of the aforementioned studies involved the use of non-salmonid species, and very little physiological data is available for freshwater species not classified as salmonids. Most of the studies cited in the aforementioned article regarding non-salmonid species were conducted prior to 1980 and communicated to the authors via second hand sources. This gap illustrates the need for data regarding this group of fish, as non-salmonid, freshwater species are increasingly being exposed to elevated suspended sediment concentrations.

Effects of Suspended Sediment on Filter Feeding Invertebrates

Forming one of the lower levels in the food chain, filter feeding invertebrates play a critical role in the continued health of communities and ecosystems. By feeding on dispersed plant matter, they concentrate energy and make it readily available for use by higher organisms. Their heightened sensitivity to disturbances has also made them ideal test organisms and the basis for many water quality regulations. Most studies regarding invertebrates and suspended sediment have involved organisms of the order *Cladocera*, though a few have branched into species of mussels (*Ostreoida and Mytiloida)* and caddisflies. Not surprisingly, these organisms are much more sensitive to suspended sediment concentrations than higher organisms such as fish. They filter water to obtain energy from the breakdown of plant matter. In the case of organisms of the order *Cladocera*, this process causes them to ingest particles of sediment that subsequently become lodged in the gut tract. Depending on the age and species of the organisms, these particles can be cleared or become lodged in the gut tract [34-37].

Numerous studies have demonstrated the process described above [35-38]. Sublethal effects have been examined more than mortality, although Kirk and Gilbert [38] showed that only 10% of juvenile *Daphnia ambigua* survived 7 days when exposed to 50 mg/L clay; adults were more robust, showing no mortality under the same circumstances. Ingestion rates of *Daphnia pulex* were also shown to increase at 10 mg/L clay, then subsequently decrease at 50 mg/L and 100 mg/L suspended clay. A separate study involving *D. pulex* found similar results, reporting a decrease in filtering rates from 4 ml animal^{-1} hr^{-1} at 0 NTU to 1.5 ml animal^{-1} hr^{-1} at 60 NTU [36]. A decrease in assimilation efficiency from 80% to 28% over 0-60 NTU and 0.45-1.0 x 10^5 algal cells/ml was also reported for *D. pulex*. Clearance rates for *Daphnia carinata* were also shown to decrease from 24 ml *Daphnia*$^{-1}$ day^{-1} to 2 ml *Daphnia*$^{-1}$ day^{-1} over 0-100 NTU [34]. It should be noted that the decrease reached a minimum of 2 ml *Daphnia*$^{-1}$ day^{-1} at roughly 30 NTU and remained depressed through the 100

NTU treatment. Other effects on cladocerans included increased weight [39], sediment abrasion of the exoskeleton , and mortality from depleted oxygen levels brought on by resuspension of reduced sediment [34,40].

Other species of filter feeding invertebrates seem to be affected differently than cladocerans, again showing the unique sensitivity of this order to environmental disturbances. Rotifers have been shown to be more resilient than cladocerans with regard to suspended sediment [34,38]. Rotifers are selective feeders whereas cladocerans are nonselective. This difference results in rotifers frequently rejecting food items in the presence of suspended clay. This process can lead to population dominance shifts from cladocerans to rotifers following exposure [38]. Effects have also been shown in the green-lipped mussel *Perna viridis* exposed to silt and sand concentrations ranging from 250-1000 mg/L for 14 days [41]. Qualitative examinations of the gills showed extensive damage in the highest exposure (1000 mg/L), and significant damage in lower exposures (500 and 750 mg/L). More importantly, damaged cilia were not repaired or replaced when the organisms were transferred to clean media for up for 28 days. This suggests that gill function in the green-lipped mussel may be permanently impaired following exposure to suspended solids. Other studies involving bivalves have shown similar effects as those noted for other filter feeders including decreased clearance rates, oxygen consumption, and growth [41-46]. A study involving the shrimp *Penaeus japonicus* showed impaired ion regulation as evidenced by a decrease in circulating Na^+ and Cl^- concentrations and increases in Na^+/K^+-ATPase activity following exposure to 35 and 65 NTU (152 and 910 mg/L) for three weeks [47]. Although no visual gill damage was observed, effects were concluded to have come from similar processes as described above including abrasion of gill filaments, smothering of gill filaments with sediment and mucus, and a hindered oxygen transfer across the gills.

INTRODUCTION

Suspended sediment is a natural and necessary component of aquatic ecosystems. However, anthropogenic activity within watersheds significantly increases suspended sediment concentrations, especially during storm events, and this can be harmful to aquatic organisms. Previous studies have focused on the effects of excessive suspended sediment on mortality thresholds and habitat alteration. More recently, researchers have reported that fish and some filter feeding invertebrates may be significantly affected at much lower concentrations than those that are lethal. Some researchers have found that the dose-response relationship for suspended sediment may depend on physical properties of the particles themselves, such as size and angularity [18].

A considerable body of literature was accumulated in the 1980s with respect to the effects of suspended sediment on fish. Much of this work focused on salmonids; thus, there are limited data concerning the effects of suspended sediment on warm water fish species. These species inhabit many areas that have come under increasing pressure from urban development. Even slight increases in suspended sediment concentrations may hinder reproduction and feeding behaviors of fish as well as induce physiological stress that may further impact behavior [4,18-22].

Suspended sediment also exerts a heavy influence on filter feeding invertebrate communities. These organisms often serve as an integral level in the aquatic food chain and are necessary for energy transport throughout the system. Effects of suspended sediment on invertebrates of the order *Cladocera* have been well documented, and low levels (<50 mg/L) of suspended sediment can cause significant damage to a community in the form of decreased growth, reproduction, and survival [35-37]. Suspended sediment can also cause shifts in dominant species [38] that can cause subsequent effects on predator populations. Effects are also seen in other filter feeding organisms such as bivalves. Abrasion of the gills has been documented in the green-lipped mussel with effects on ion regulating enzyme activity [41].

Increases in suspended sediment beyond normal concentrations have become the focus of recent research and guideline development. BMP's have been designed and put in place to minimize the amount of sediment transported into streams as a result of landscape disturbances. Currently, individual states are responsible for setting and enforcing their own suspended sediment criteria. Differences in standards and enforcement actions have made this an unreliable network and the federal government has recently compiled a review that will hopefully make suspended sediment criteria more cohesive. One of the major data gaps for this review is the amount of data concerning warm water fishes. This study was designed to assess the effects of suspended natural clay on the common test organisms *P. promelas and D. magna* and compare these effects, if any, to effects observed from defined clay.

OBJECTIVES

The goal of this study was to characterize the responses of *Daphnia magna* and *Pimephales promelas* (fathead minnows) to acute exposures of suspended clays. A secondary goal was to compare the effects of isolated natural clay-size particles with those of defined clay powders. These goals were accomplished by achieving the following objectives:

Daphnia magna:

1.) Characterize mortality response to suspended clay exposure.

2.) Characterize the response of growth and reproduction following exposure to suspended clay using body length and days to gravidity.

3.) Characterize clearance process of clays from the gut tract.

4.) Compare responses between defined and natural clays.

Pimephales promelas:

1.) Characterize the response of gill Na^+/K^+ ATPase, whole-body sodium, and percent body moisture to suspended clay exposure.

2.) Characterize the effect of duration of suspended clay exposure on the aforementioned variables.

3.) Compare responses between defined and natural clays.

MATERIALS AND METHODS

Sediment Collection and Preparation

Sediment was collected from the banks of Lost Creek in the Saluda River watershed within the Piedmont ecoregion of South Carolina. Sediment was collected in 5-gallon plastic buckets and transported back to the Institute of Environmental Toxicology, Clemson University. The clay-sized fraction (<2 µm) was collected using gravimetric techniques following Stoke's Law and assuming a particle density of 2.65 g/cm^3. This fraction was then concentrated by centrifuging at 3000 *g* for 30 minutes using an IEC Centra GP8 centrifuge. Centrifuged pellets were resuspended in deionized water and the solution was sonicated for one hour before concentration was determined using Standard Method 2540 D [17]. Pall Metrigard glass fiber filters were used as the filtering media with particle retention of 0.5 µm. These natural clay-sized particles (LC) were analyzed by x-ray diffraction analysis (XRD) (The Mineral Lab, Lakewood, CO, USA) and were found to be primarily kaolinite (60%) (Table 1).

Stock solutions of defined clays were prepared by mixing clay powder in deionized water on a stir plate overnight followed by sonication and concentration determination described above. Defined clay powder was assumed to be <2 µm and pure. Kaolinite (KN) clay powder was purchased from VWR International (CAS: 1332-58-7) and Montmorillonite (MN) clay powder was purchased from Ward's Natural Science, Rochester, NY, USA.

Mineral Name	Chemical Formula	Approx. Wt %
Kaolinite	$Al_2Si_2O_5(OH)_4$	60
Gibbsite	$Al(OH)_3$	10
Goethite	FeO(OH)	12
Quartz	SiO_2	<5
Mica/illite	$(K,Na,Ca)(Al,Mg,Fe)_2(Si,Al)_4O_{10}(OH,F)_2$	5
K-feldspar	$KAlSi_3O_8$	<3?
Plagioclase feldspar	$(Na,Ca)Al(Si,Al)_3O_8$	<3?
Smectite	$(Ca,Na)_x(Al,Mg,Fe)_4(Si,Al)_8O_{20}(OH,F)_4 \cdot nH_2O$	<5?
"Unidentified"	?	<5

Table 1: XRD analysis of clay fraction (<2 μm) Lost Creek sediment. Question marks indicate uncertainty about mineral identification and amount.

Toxicity Tests

P. promelas

Adult fathead minnows (age 6-9 months) were purchased from Aquatic Biosystems, Inc (Aquatic Biosystems, Fort Collins, CO, USA). Organisms were maintained in a 100-gallon flow through raceway containing dechlorinated tap water. The laboratory was maintained at 25 ± 2° C with a 16:8h, light: : dark photoperiod. Organisms were fed TetraMin (Tetra, Blacksburg, VA, USA) flake food once daily. Ammonia and nitrite levels were measured weekly and were consistently below 0.25 mg/L.

Test setup consisted of twenty 3.5 L plastic funnels purchased from US Plastics, Lima, OH. Funnels were suspended from wooden racks supported by wire shelves. Individual air-lines were secured through rubber stoppers in the base of the funnels to maintain clay suspensions. Test setup is shown in Figure 4. Funnels were filled with 3 L of dechlorinated tap water before the addition of the appropriate volume of stock sediment solution to yield concentrations of 0, 50, 100, 250, 500, and 1000 mg/L suspended clay. All exposures were conducted in quadruplicate. One adult fish was placed in each funnel and the funnel was covered with a 12x8 inch piece of plexiglass to mitigate evaporation and ensure fish did not jump from the funnel. Exposure durations were 12, 24, 96, and 168 hours. Water temperature for all trials was 22 ± 3°C and pH was 7.5 ± 0.5. Complete water changes were performed every 48 hours and water quality (pH, temperature, dissolved oxygen, TSS, ammonia) taken before and after each renewal. Fish were anesthetized in pH buffered solution of 200 mg/L MS-222 until gill movement ceased. Gills were then dissected and placed in 100 ml SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM Imidazole) and frozen at -80 °C until analysis of gill Na^+/K^+ ATPase by the method described in McCormick et al [48]. Protein determinations were made using a BCA™ Protein Assay Kit (Pierce, Rockford, IL, USA). Carcasses were patted dry and placed in 50 mL centrifuge vials and weighed to obtain initial weights. Final weights were obtained after drying in an oven for 96 hours at 90°C. Dried fish were then digested in 20 ml of 50% concentrated nitric acid (trace metal grade, Fisher Scientific, Pittsburg, PA, USA) in a 100 °C water bath for one hour. Each sample was then diluted to 40 ml by adding 4 ml of 20 g/L K^+ (as KCl) and 16 ml of deionized water. Samples were then diluted 1:40 and analyzed for sodium on an atomic absorption spectrophotometer (Perkin-Elmer AA800, Perkin-Elmer, Shelton, CT, USA) via the flame emission mode.

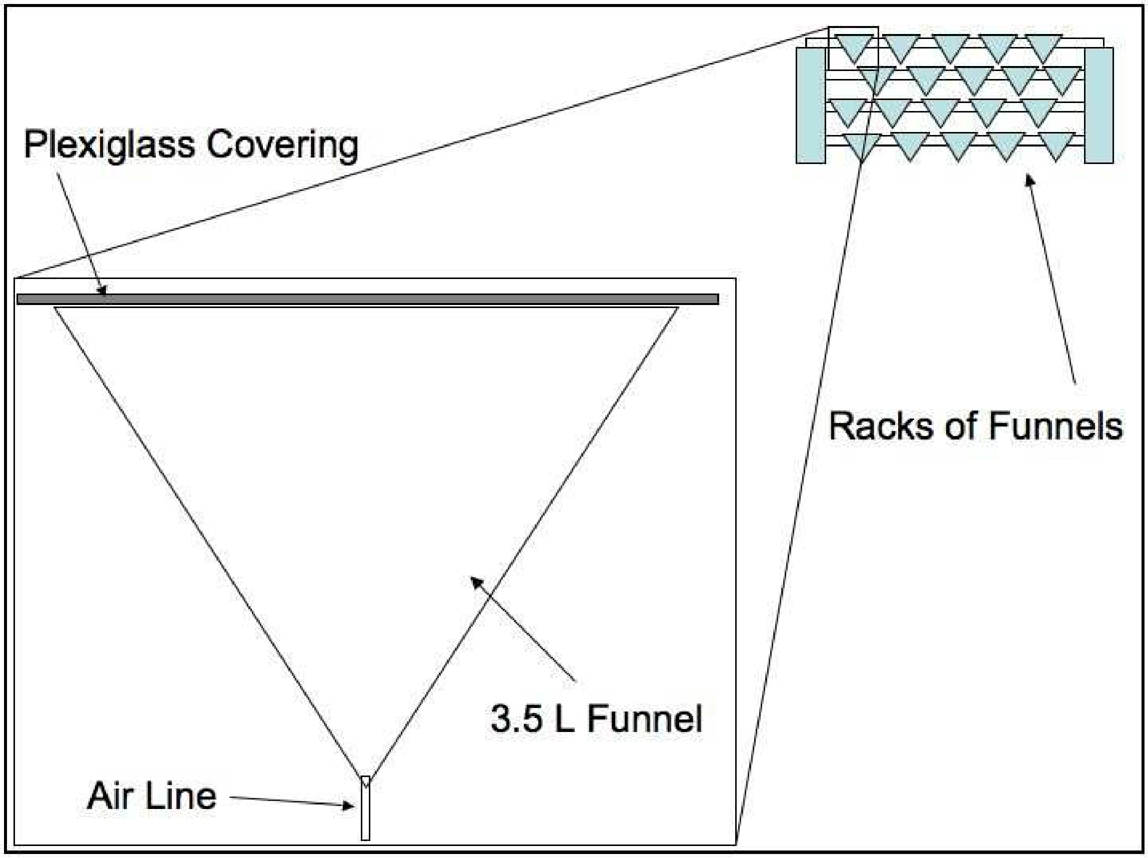

Figure 3: Diagram showing test setup for the *P. promelas* trials.

D. magna

Daphnids used in this study were cultured at the Institute of Environmental Toxicology, Clemson University. Organisms were cultured in 1 L beakers containing 600 ml of reconstituted soft water (14.4 g $NaHCO_3$, 9.0 g $CaSO_4$, 9.0 g $MgSO_4$, 0.62 g KCl in 300 L deionized water) with a hardness of 45 ± 5 mg/L as $CaCO_3$ and an alkalinity of 35 ± 5 mg/L as $CaCO_3$. These organisms are found naturally in high hardness waters but are routinely cultured in house in reconstituted moderately hard water with a hardness of 80-100 mg/L as $CaCO_3$ and an alkalinity of 60-80 mg/L as $CaCO_3$. Following a three-week acclimation period in which the organisms were systematically transferred to culture water of gradually decreasing hardness and alkalinity organisms were observed to be healthy and following normal growth and reproduction patterns. Low hardness and alkalinity were required in order to minimize clay aggregation in the test chambers due to interactions between the clay particles and dissolved ions. Organisms were fed a stock solution of the unicellular green algae *Selenastrum capricornutum* daily to maintain a concentration of 300,000 cells/ml. Daphnids were also fed a yeast-cerophyll-trout chow combination at half the volume of added algal stock.

Organisms were exposed using 4 L plastic beakers containing two-inch stir bars on stir plates. Individual 72-hour old *D. magna* were placed inside cylindrical glass test chambers. Chambers were constructed of 22 mm ID (40 mm tall) fused quartz glass tubing (Technical Glass Products, Painesville Township, OH, USA) capped on both ends with 150 µm Teflon mesh (Rickly Hydrological Company, Columbus, OH, USA). Teflon mesh was fastened on lower end of chamber via aquarium safe silicon and secured on top with a 26 mm ID (10 mm tall) glass ring. This was done to allow easy access to exposure chambers during testing. Eight chambers were placed in a modified test tube rack that was then suspended in an exposure beaker. The stir plates and bars kept the particles in solution by circulating water within the test chamber in the pattern depicted in Figure 4. A dye study was conducted prior to test initiation to ensure adequate mixing within the chamber. Seven-day mortality, growth, and reproduction tests were conducted with all three clay types. For the mortality tests, stock KN solution was added to make concentrations of 0, 25, 50, 100, and 200 mg/L, stock MN added to make concentrations of 0, 5, 15, 30, 50, and 75 mg/L, and stock LC added to make concentrations of 0, 25, 50, 75, 100, and 150 mg/L. Sublethal tests were conducted at 50 mg/L. During pulsed exposure, organisms were exposed to LC for 48 hours, transferred to clean media for the remaining 120 hours, and growth was measured daily. Test media was renewed every 24 hours for all test types. All tests were fed *Selenastrum capricornutum* at a rate of 150,000 cells/ml, or roughly ½ the culture concentration. All exposures were performed in duplicate, for a total of 16 organisms per treatment. Water quality (pH, temperature, dissolved oxygen, TSS) was taken at initiation, before and after each renewal, and at termination. Photoperiod for all test was 16:8 hr light-dark cycle. Due to the relatively small size of the test beakers, light penetration was not expected to affect any of the endpoints.

During the bioassays, live organisms were counted daily. The test tube rack was removed from the test beaker and set in a bowl of clean media. Test media were renewed and racks returned to the beakers. The same process was used for the sublethal exposures but organisms were measured from the base of the tail to eye using a dissecting scope equipped with a micrometer and gravidity noted before renewal.

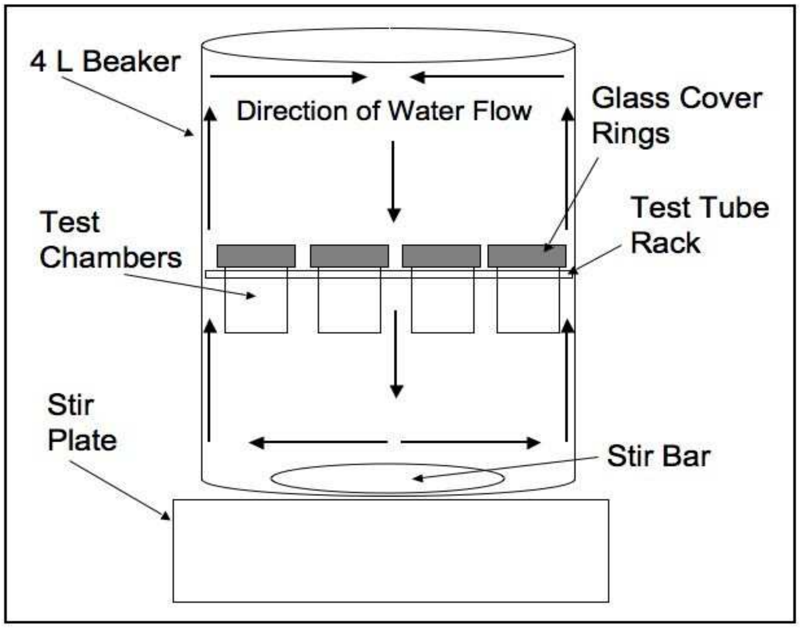

Figure 4: Diagram depicting water flow and test chamber setup for *D. magna* trials.

Water Quality Analysis

All TSS samples were analyzed according to Standard Method 2540 D (APHA 1998). Pall Metrigard glass fiber filters were used as the filtering media with particle retention of 0.5 µm. Temperature and dissolved oxygen were measured using a YSI Model 85 handheld probe (Yellow Springs Instruments, Yellow Springs, OH, USA). An Orion 710A+ pH meter was used to measure pH and ammonia, with an Orion 9512 probe attached to measure the latter.

Statistical Analyses

Dose-response slopes were tested using regression analysis and homogeneity of slopes. 7-d LC_{50} values were calculated using Trimmed Spearman-Karber techniques with ToxStat. Differences in daphnid body length and days to gravidity were tested by one-way ANOVA. Significance for all tests was at $p \leq 0.05$.

All variables for the fish trials (whole-body sodium, Na^+/K^+-ATPase, and % body moisture) were tested by two-way ANOVA. If significant differences were found, the variables were then tested separately by one-way ANOVA and the Tukey-Kramer HSD test ($p \leq 0.05$). All of the data from the fish trials was also normalized as percent of controls by dividing mean control values for each time point by mean exposure values and multiplying by 100.

RESULTS

D. magna

Due to particle adhesion onto the mesh and testing chambers, TSS concentrations declined over 24 hour intervals for both the lethal and sublethal tests. Exposure concentrations were calculated as the average difference between initial and final values over the test period. All results are based on these calculated exposure concentrations. Suspended clay caused dose-dependent mortality (Figure 5). The slopes of the response curves in Figure 5 were compared using homogeneity of slopes and linear regression techniques. Significant differences were found between MN and LC ($p=0.0294$) and LC and KN ($p=0.0304$). MN and KN were not statistically significant ($p=0.0973$). Trimmed Spearman-Karber was used to calculate 7-d LC_{50} values for *D. magna* exposed to the three clay sources. Values were 5.17 (95% CI 2.81,9.54), 51.02 (31.25, 83.31), and 74.51 (65.08, 85.3) mg/L for MN, LC, and KN, respectively.

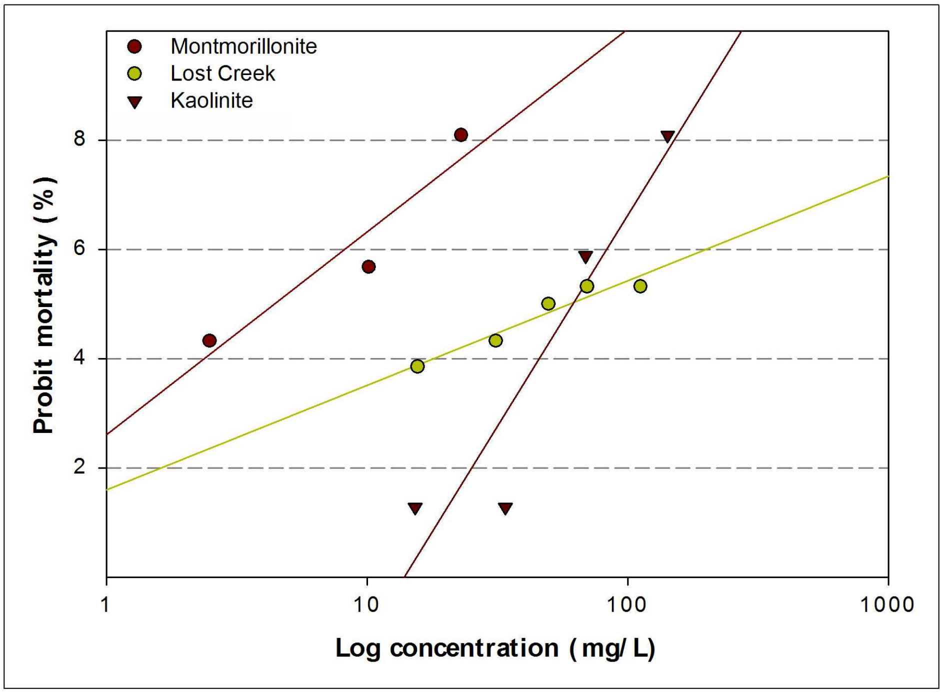

Figure 5: Mortality response of *D. magna* exposed to suspended clays for 7 days.

All sediment exposures reduced growth after seven days compared to controls (Figure 6) ($p < 0.05$ all treatments). Differences in the amount of particles adhering to the test chambers caused the concentrations of the sublethal test to vary between treatments. Actual exposure concentrations are noted in Figure 4. After 48 hours, daphnids in all exposures showed reduced growth compared to the controls ($p < 0.05$ all treatments). After 72 hours, daphnids in each exposure were statistically different from the other exposures and controls ($p < 0.05$ all treatments). There was no significant growth after 120, 120, 96, and 24 hours for the controls, KN, LC, and MN, respectively. This pattern of toxicity is consistent with the mortality results (MN>LC>KN). Growth rates were also calculated during the growth test and are shown in Figure 7. Daphnids exposed to MN particles were exhibited decreased growth rates compared to controls at 48 hours ($p = 0.0403$) and remained depressed through 120 hours. LC exposed organisms showed decreased growth rates from 72 to

120 hours ($p = 0.0005$). KN only showed a significantly different growth rate than the controls at 120 hours ($p = 0.0032$). There were no significant differences in growth rates in any treatment at 144 and 168 hours. Mean growth rates are shown in Table 2.

	Particle Source			
Time	**CTL**	**KN**	**LC**	**MN**
48	0.201	0.125	0.158	0.048
72	0.181	0.057	0.173	0.000
96	0.117	0.035	0.120	0.005
120	0.163	0.051	0.063	0.140
144	0.029	0.046	0.072	0.000
168	0.080	0.004	0.034	

Table 2: Mean growth rates of *D. magna* during the sublethal exposure.

D. magna exposed to LC for 48 hours then transferred to clean media showed decreased growth (Figure 8). Organisms were still significantly smaller than controls after five days of recovery ($p < 0.05$). Clearance of the gut tract was qualitatively assessed by a series of pictures shown in Figures 9-13. All pictures are of the same organism. Complete gut clearance occurred within 30 minutes after transfer to clean media.

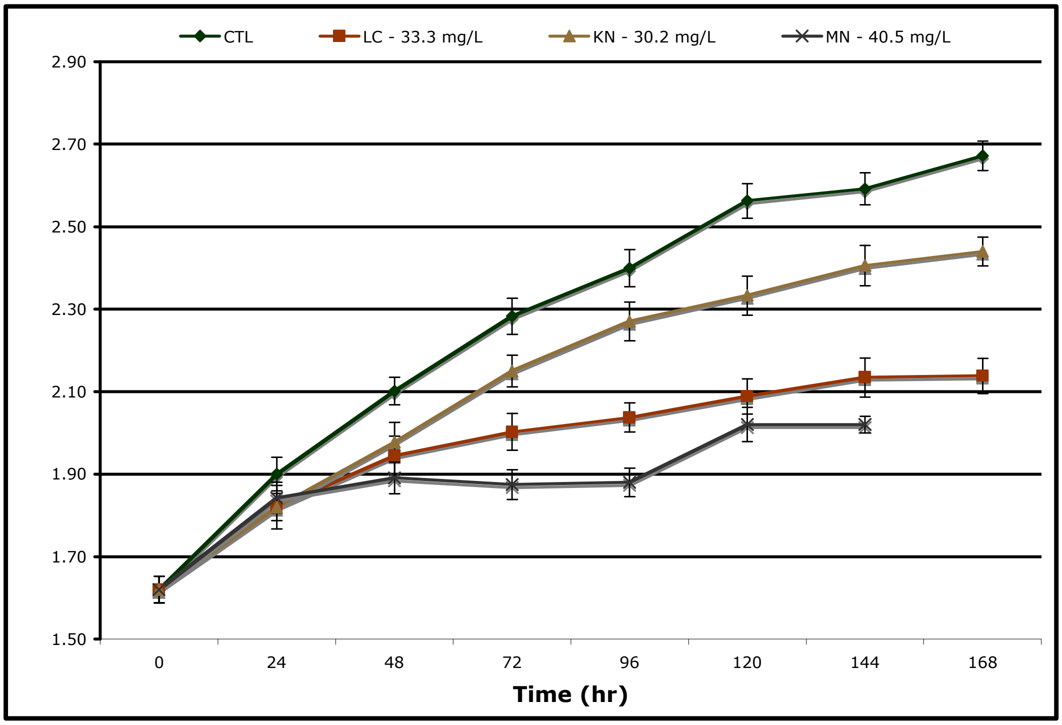

Figure 6: Mean body length of *D. magna* over time. Actual exposure concentrations shown in legend. Exposures were statistically different from controls at all time points > 24 hours. Error bars represent standard error.

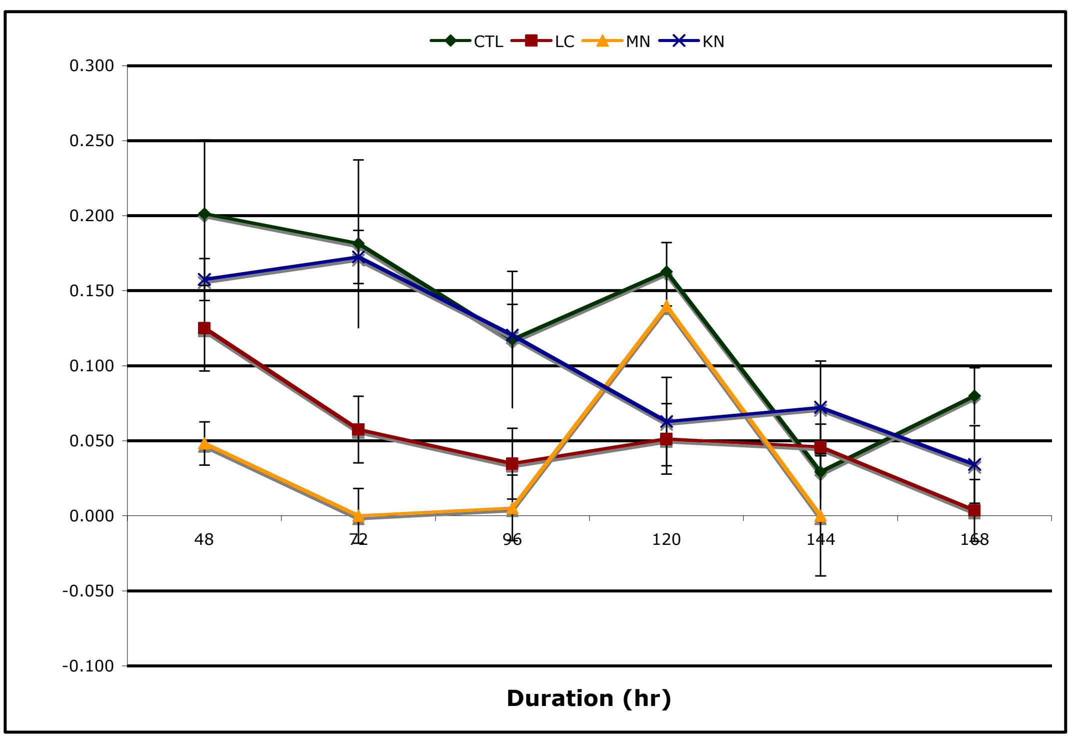

Figure 7: Growth rates of *D. magna* over exposure duration.

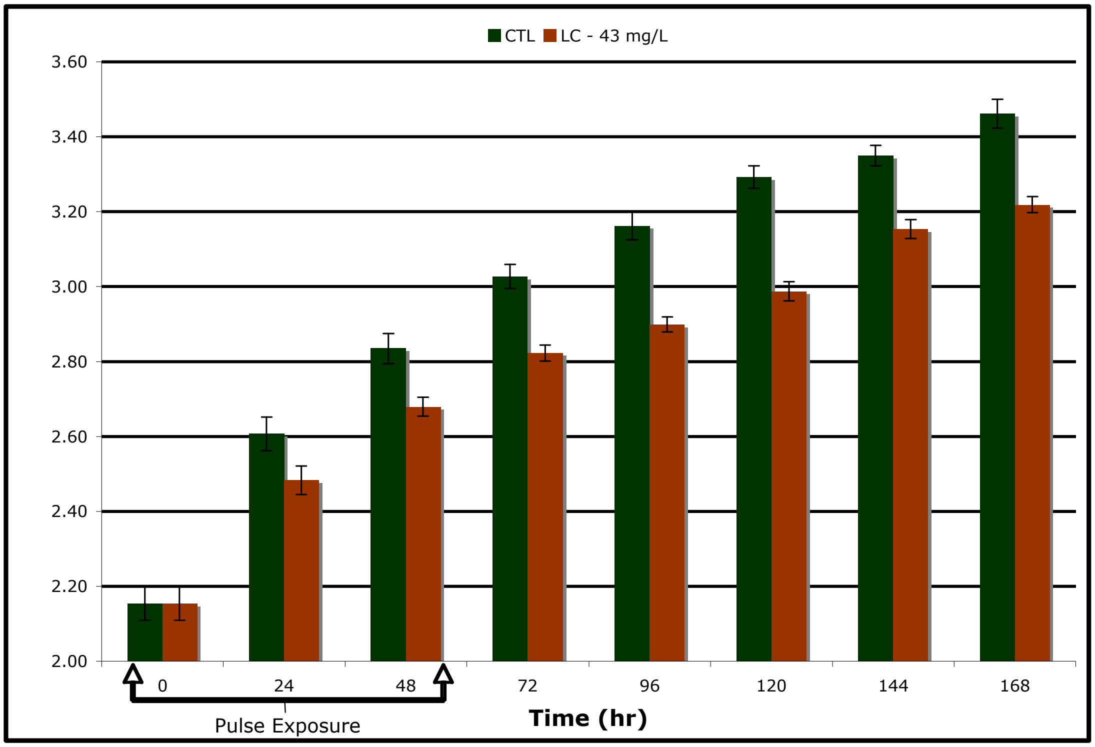

Figure 8: Mean body lengths of *D. magna* over time. Arrows indicate pulse exposure of clay-sized particles. Exposures were statistically different from the control group at all time points > 0 hours ($p < 0.05$).

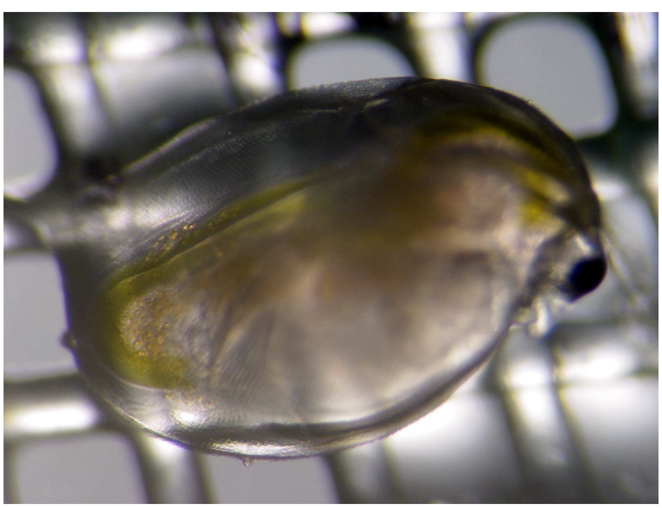

Figure 9: Control organism 1 minute after removal from test beaker.

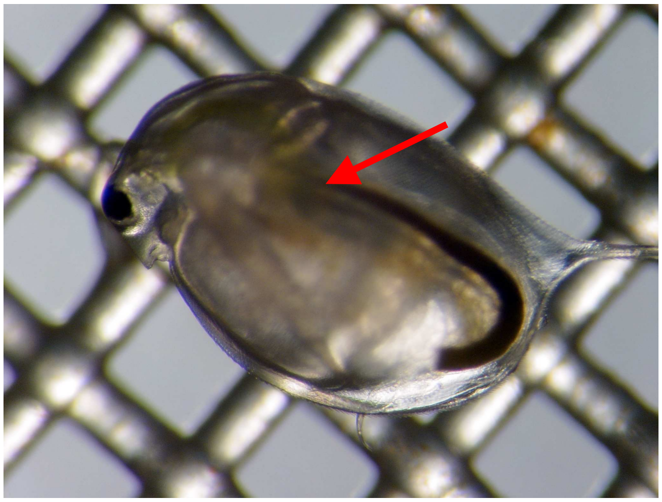

Figure 10: Exposed organism 1 minute after removal from test solution. Note gut has already been partially cleared (arrow).

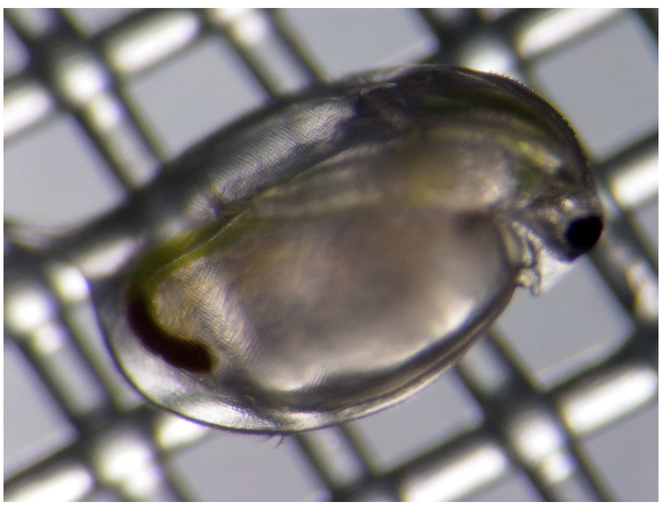

Figure 11: Exposed organism 5 minutes after removal from test solution.

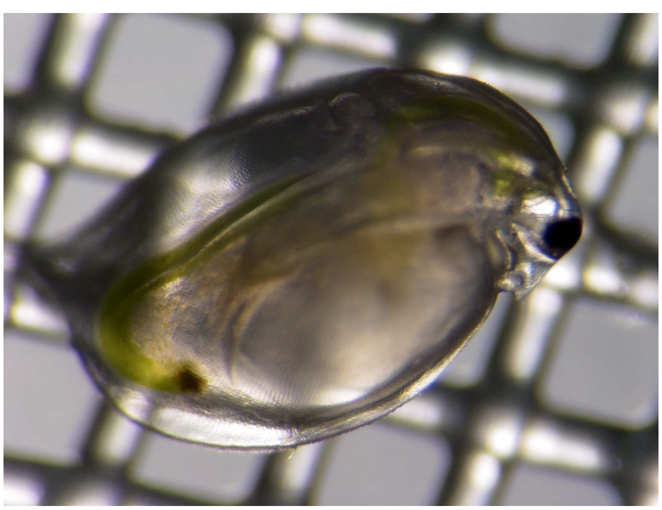

Figure 12: Exposed organism 15 minutes after removal from test solution. Only a small plug of sediment remains in the gut tract.

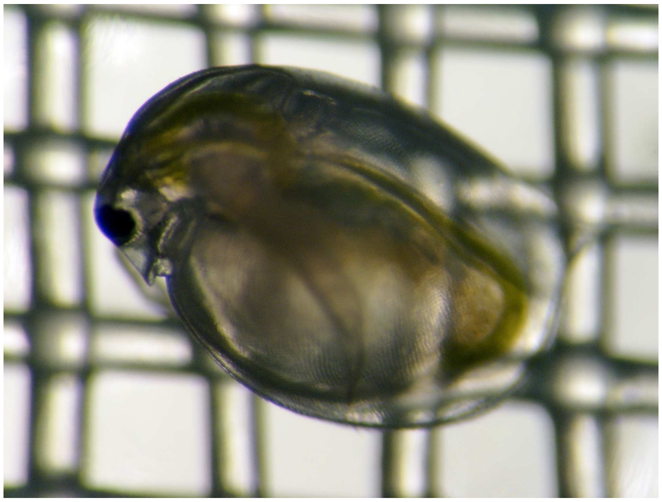

Figure 13: Exposed organism 30 minutes after removal from test solution. Gut tract has been cleared.

The LC exposure increased the number of days to gravidity (DtG)(Table 3). Numbers represent the age each daphnid became gravid, keeping in mind the test was initiated on day three of each daphnia's life. The average number of days for *D. magna* to become gravid in the presence of LC was significantly higher than the controls and KN ($p < 0.05$). DtG was not significantly increased in the KN exposure compared to the controls ($p > 0.05$). This is similar to the growth data that indicated decreased growth overall in the KN compared to the control treatment, but growth still occurred for the same period of time in both treatments. Due to mortality, reproduction data was not obtained for the MN exposure.

Sediment	Replicate																n	Mean
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
CTL	5	6	5	5	6	5	5	6	5	7	5	6	4	6	5	†	15	5.40
KN	4	6	5	5	6	5	5	6	5	5	6	6	6	6	6	4	16	5.38
LC	4	11	8	11	5	11	11	†	†	11	†	11	4	†	5	11	12	8.58
MN	†	†	†	†	†	†	†	†	†	†	†	4	†	†	†	†	1	N/A

Table 3: DtG for *D. magna* exposed to suspended clay for 7 days. Numbers since birth. Organisms that survived the test but never became gravid were assigned a value of 11. † indicates organisms did not survive test and never became gravid.

<u>*P. promelas*</u>

Similarly to the daphnid results, circulating suspended solids concentrations were altered from nominal concentrations over the course of testing. Actual concentrations were measured, and all showed a 5-20% reduction from nominal concentrations. All results are presented in the form of actual concentrations to make the results less complicated.

There was no significant effect of LC or KN on WBNa, Na^+/K^+-ATPase, or % BM by concentration or duration ($p>0.05$) Exposure to MN elicited a significant WBNa response as a function of concentration and duration ($p < 0.0001$). Figure 14 shows the relationship between duration and WBNa for all exposure concentrations. Due to the trend in the controls, WBNa data was normalized to percent of controls and is shown in Figures 15 and 16 by duration and concentration respectively. WBNa generally increased with increasing exposure duration and generally decreased with increasing concentration.

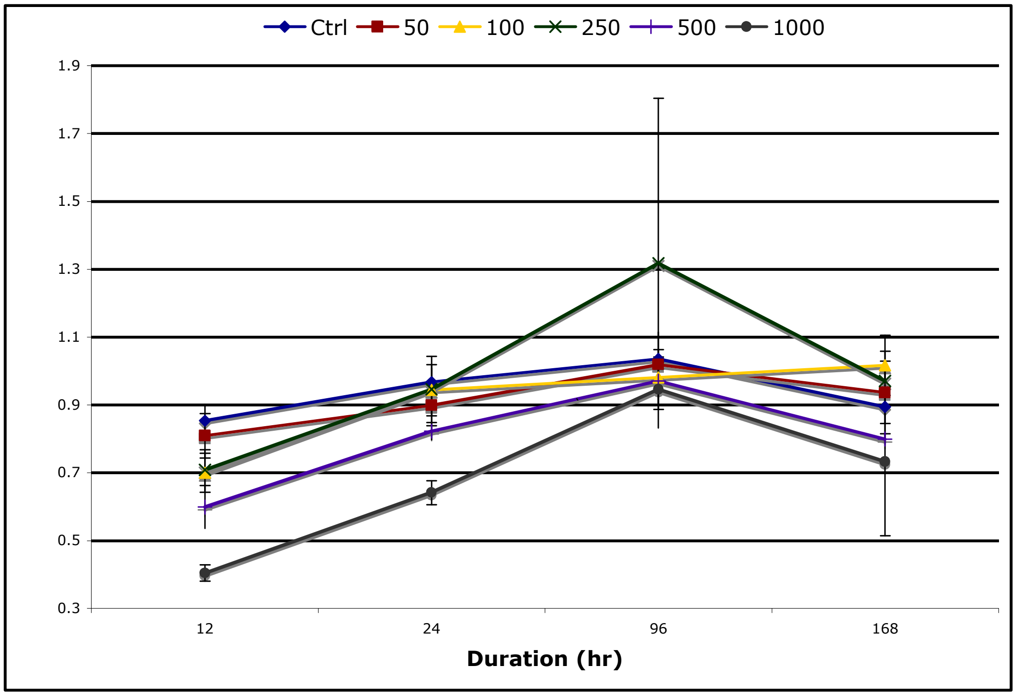

Figure 14: WBNa versus MN exposure duration over exposure concentrations. WBNa generally increases with increasing exposure duration through 96 hours then decreases at 168 hours.

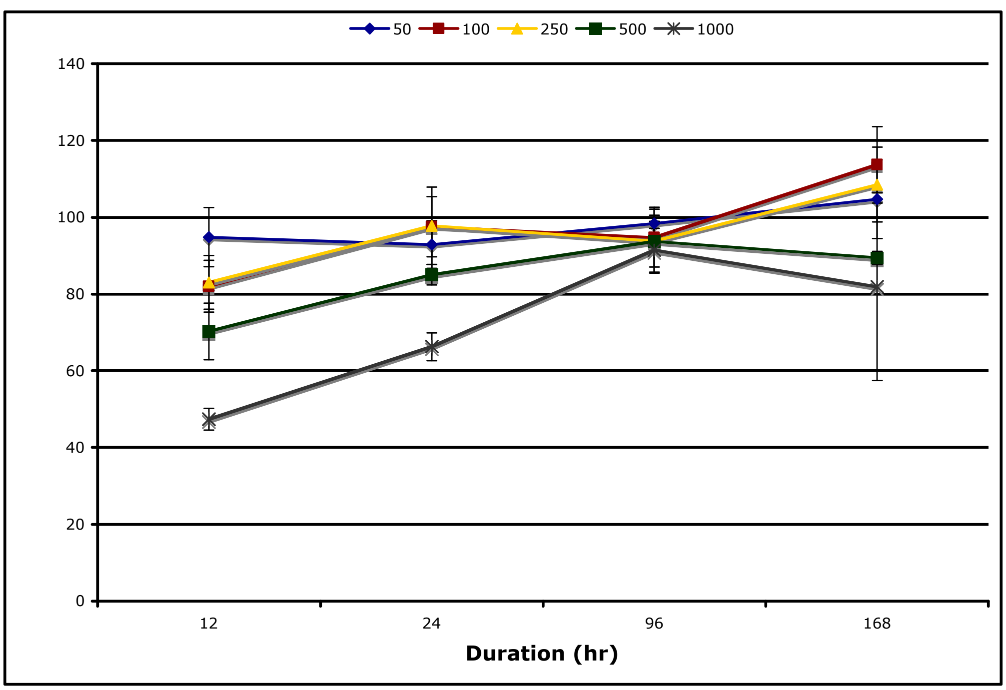

Figure 15: WBNa as a function of MN exposure duration normalized to control data. WBNa is again seen to generally increase as exposure duration increases.

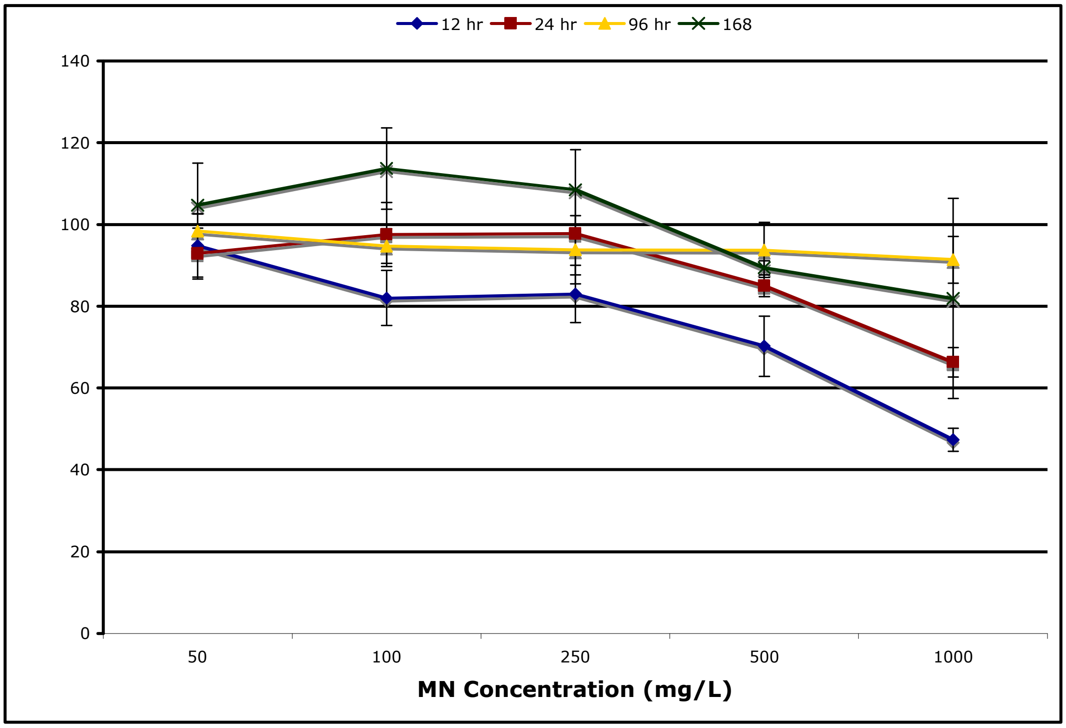

Figure 16: WBNa as a function of MN exposure concentration normalized to control data. WBNa generally decreases with increasing exposure concentration.

There was a negative correlation between MN exposure duration and Na^+/K^+-ATPase activity ($p = 0.0046$). Figure 17 shows the decrease in activity through 96 hours then leveling. Concentrations of 500 mg/L and 1,000 mg/L significantly increased Na^+/K^+-ATPase activity at 12 hr ($p = 0.0053$). Concentrations of 100 and 250 mg/L also significantly decreased Na^+/K^+-ATPase activity at 12 hr. There were no statistical differences in any other time points for Na^+/K^+-ATPase activity. All concentrations caused an non-significant increase in % BM relative to controls by 12 hours, a decrease between 12 to 24 hours, and a slight increase through 168 hours (Figure 19). Data was again normalized to control values. Although a two-way ANOVA analysis showed a duration effect on % BM during MN exposure ($p = 0.0023$), individual ANOVA analysis of % BM values show no statistical differences from control values at any time point ($p > 0.05$).

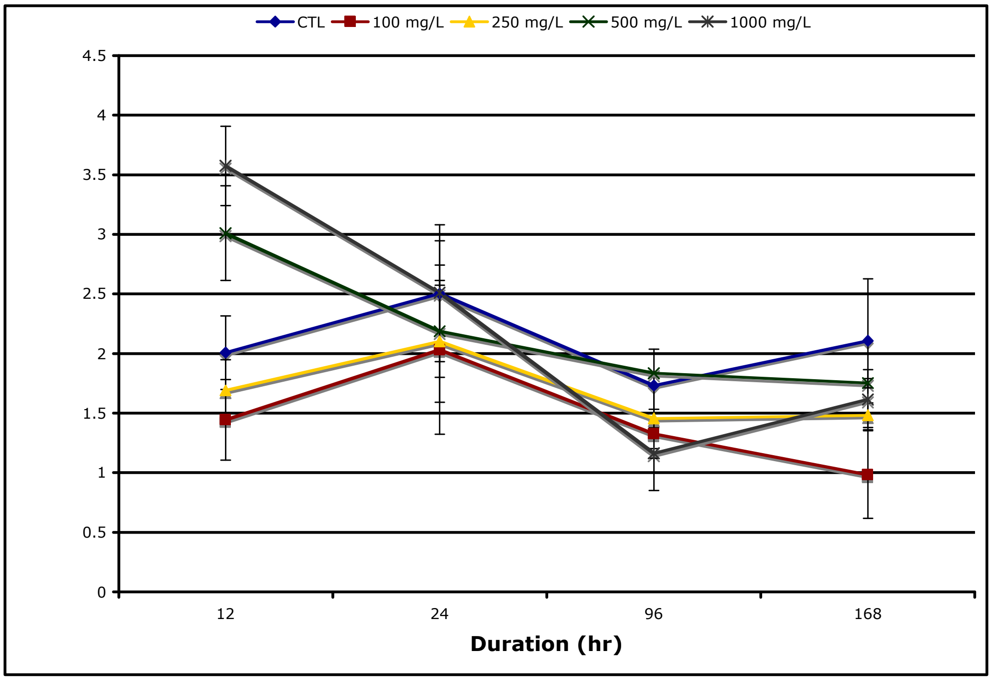

Figure 17: Na$^+$/K$^+$-ATPase activity as a function of MN exposure duration. Exposure activity is elevated during short exposures at high concentrations.

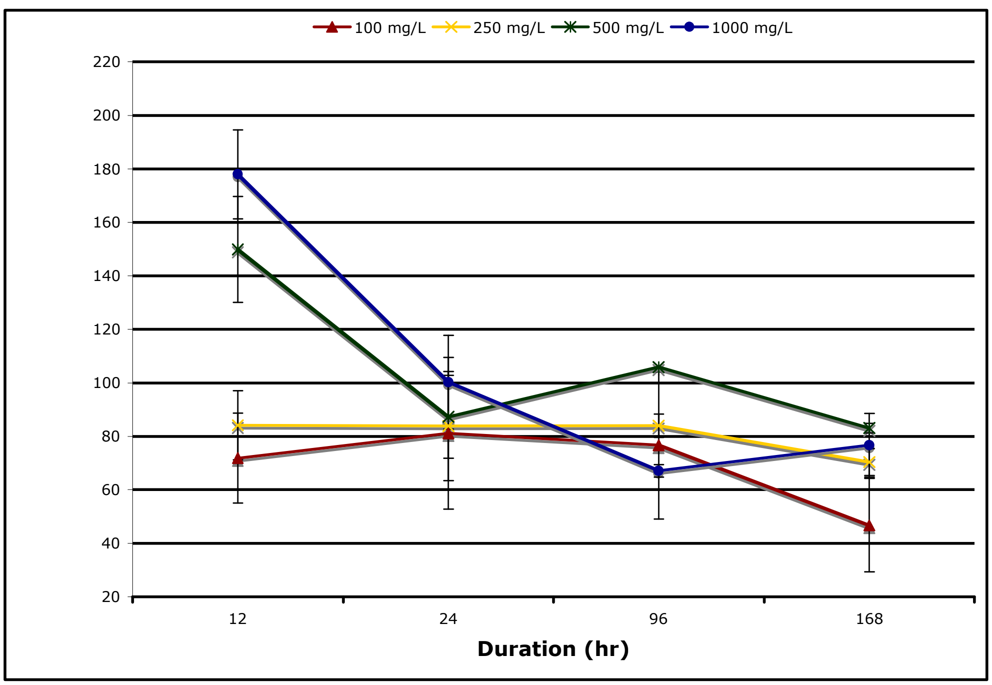

Figure 18: Na$^+$/K$^+$-ATPase activity as a function of MN exposure duration, normalized to control data. Exposure activity is elevated during short exposures at high concentrations.

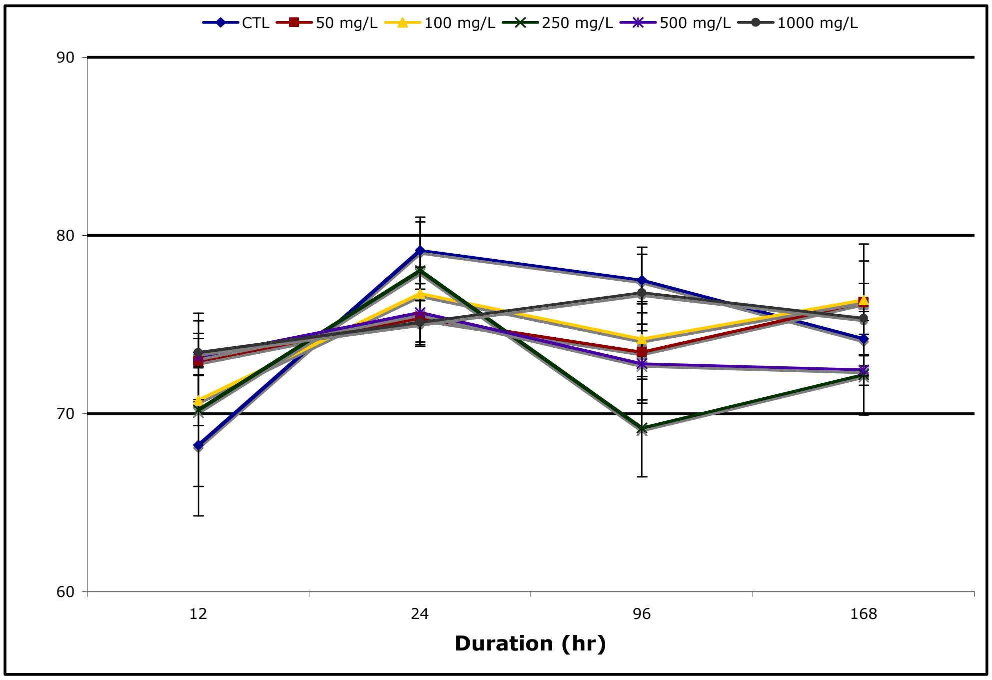

Figure 19: Percent body moisture as a function of exposure duration. No statistical trends were found.

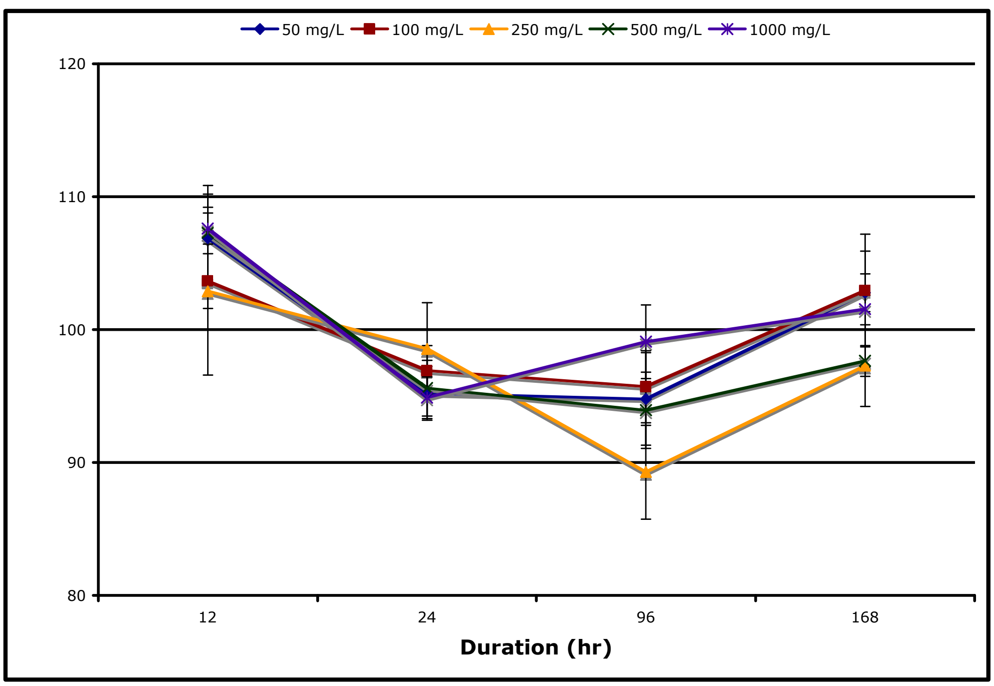

Figure 20: BM % of controls as a function of MN exposure duration, normalized to control data. % BM decreases from 12 to 24 hours relative to controls, then increases through 168 hours. No data points were statistically different from control values.

DISCUSSION

All three clay particle sources caused dose dependent mortality in *D. magna* (Figure 5). Although the concentrations used were relatively low (<100 mg/L) compared to environmental levels following storm events, mortality was high (60-100%). These results underscore previous research that also found low levels of suspended solids to be toxic to filter-feeding invertebrates [21,38]. The toxicity of the particles varied greatly with MN (7-d LC_{50} = 5.17 mg/L) being most toxic while KN (7-d LC_{50} = 74.51 mg/L) was least toxic. The slopes of the mortality curves showed significant differences. Most importantly, the slopes for the MN and KN response curves were statistically similar. This shows that although KN is less toxic in terms of the LC_{50} value, toxicity is exhibited quickly once concentration begins to increase. The LC clay fraction was approximately 60% kaolinite (Table 1) and its LC_{50} value (51.02 mg/L) falls between the MN and KN values, but much closer to the KN value. Drawing conclusions on mortality responses using 7-d LC_{50} values is complicated by the similarities the slopes of the response curves. Based on the LC_{50} values, the conclusion that LC and KN behave similarly has some validity. Including the slopes of the mortality curves in this conclusion makes this predication questionable. Despite this, it is a viable conclusion that clay from one location would produce differing effects than a clay from another location. It is possible to encounter nearly pure deposits of kaolinite and montmorillonite that, if eroded, may have markedly different effects on water column invertebrates. These data have repercussions for the work recently completed by the USEPA on broad guidelines for developing suspended solids water quality criteria. *D. magna* growth and reproduction were also significantly reduced by all three particle sources (Figure 6, Table 2). These responses can be affected by both food quality and quantity [34,36]. The amount of food used in these studies was 150,000 algal cells/ml, which is roughly half the concentration fed to culture organisms. This value is high compared to similar studies (5,000 cells/ml *Cryptomonas* sp. [38]; 3,100-120,000 cells/ml [36]; 50,000 cells/ml [35]). As a result, the test organisms ingested a higher proportion of algae to clay particles during feeding than organisms in the natural environment would under similar conditions. Therefore, these results may represent best-case scenarios of clay-sized particle effects on *D. magna*. Porter et al [37] found that the appendage rate of *D. magna* decreased with increased food concentrations so it is possible that not only did test organisms receive the benefit of a higher proportion of algae to sediment, but due to slower appendage rates, actually took less sediment in than a daphnid would under natural conditions.

Growth tests confirmed the increasing toxicity of the three particle types as KN<LC<MN. Calculated growth rates showed that MN exerted the greatest effect on daphnid growth as it affected the organisms in the shortest as well as greatest length of time. Most of the organisms in the MN exposure died during the growth and reproduction tests, resulting in no data for days to gravidity for MN. Despite growth inhibition between LC and KN being statistically different from the controls as well as each other, KN did not significantly affect reproduction. This is important because although growth was suppressed by the presence of KN particles, the effect was not

great enough to inhibit reproduction in terms of days to gravidity. Conversely, daphnids exposed to LC particles showed a 1.5x increase in days to gravidity compared to the controls and KN exposure. This is a conservative estimate as daphnids that had not become gravid by the end of the test were assumed to become gravid the following day had the test continued. Therefore, 8.58 days to gravidity in the LC exposure may be lower than the actual effect caused by LC particles. The growth rates help explain why reproduction was not significantly affected as growth rates were only significantly different at 120 hours, which is usually the time at which daphnids begin reproducing. This shows that KN exposed organisms exhibited growth rates that bordered on significantly different from controls, which led to a significant reduction in total body length, but the energy loss was not great enough to significantly affect reproduction. As there was no significant difference between control and exposed organism growth rates at 144 and 168 hours, this implies that organisms may be able to recover once the exposure is terminated or, given an indefinite exposure, the result will be a population of daphnids showing stunted growth but still functioning.

A 48 hr pulse exposure of 50 mg/L LC caused significant growth inhibition in *D. magna*. Despite being transferred to clean media at 48 hours, the exposed organisms did not make up the difference in body length compared to the controls in the remaining five days of testing. Although reproduction data was not recorded, this difference might influence population dynamics if organisms are delayed in reaching a size necessary for reproduction. As shown in Figures 6-10, *D. magna* was able to clear its gut tract of sediment quickly (<30 min) after the exposure was stopped. Thus during natural pulse suspended clay exposures, such as storm events, filter-feeding invertebrates such as *D. magna* may be able to recover if the pulse does not last longer than 48 hours. Since the mechanism of suspended sediment to filter feeding invertebrates is inhibition of energy transfer in the gut tract [35,37-38] even concentrations in the 100's range are not lethal until several days after exposure. The organism has run out of energy stores at this point and is unable to assimilate more energy due to the clogged gut.

One objective of this study was to determine the similarities and differences between clay sources and organism response. If the response of an organism is based primarily on the presence of particles then any clay source would yield the same effect. Conversely, if composition plays a role then effects levels may vary. This hypothesis was tested in order to determine the plausibility of bringing a certain level of standardization to suspended solids toxicity testing. This would allow regulators to develop a much more cohesive and coherent dataset with regards to suspended solids in order to effectively protect aquatic habitat and life. However, given the wide range of LC_{50} values determined in this study (5-74 mg/L), the similarities between mortality curve slopes and particle types, and the differing effects on days to gravidity and growth, it appears that the response of *D. magna* is affected by the source of clay-sized particles. This suggests suspended solids guidelines may require development based on the composition of soils comprising individual watersheds.

Suspended clay showed limited effects on *P. promelas* at the concentrations and durations tested. Only the MN source produced significant effects, however the effects produced may be ecologically significant. The duration effect on WBNa

(Figures 14 and 15) seems counterintuitive to the duration effect on Na^+/K^+-ATPase (Figure 17 and 18), as the enzyme transports sodium into the organism. Based on this, one would expect increased WBNa concentrations to be indicative of an increase in enzyme activity. Au et al [20] reported similar results in the green grouper (*Epinephelus coioides)*. They found that although Na^+/K^+-ATPase was depressed after exposure to 2,000 mg/L for six weeks, the osmoregulatory status, measured as plasma osmolarity, of the organism was unchanged. A corresponding increase in chloride cell density, the site of Na^+/K^+-ATPase action, was thought to be responsible for this phenomenon at higher suspended solids concentrations. It was concluded that the increase in chloride cell density allowed the fish to maintain ionic balance up to the highest exposure concentration. It is possible a similar process took place in *P. promelas* during this study resulting in an increase in enzyme capacity to maintain and even increase WBNa concentrations.

The increase in control WBNa concentrations depicted in Figure 14 has been noted in studies investigating copper exposure to larval fathead minnows [49-50]. This increase in control WBNa concentrations has no obvious explanation, but could be the result of stress associated with testing. Zahner et al [49] and Vangenderen [50] also documented greater WBNa losses during the initial 12 hours of copper exposure. Although this phenomenon was concluded to be the result of limited binding sites for the copper ion, that cannot be the case in this study. It may be possible, assuming the above inference regarding chloride cell density is correct, that it takes several hours to increase chloride cell density along the gill surface. This would explain the smaller effect of suspended clays on WBNa concentrations as exposure duration increases and might also help explain recovery from copper exposures after the copper source has been removed. Data for % BM offered no hints as to the effect on whole organisms osmoregulatory capability. Although a slight duration effect was shown by two-way ANOVA, individual % BM values were not significantly different from the controls at any concentration or duration.

Unlike the results for *D. magna*, which showed a strong dependence on concentration, *P. promelas* was more affected by duration of exposure than concentration. This is not surprising, as the concentrations were designed to fall in the low range of environmental concentrations found in the upstate of South Carolina. Fish species having a 24 hr LC_{10} less than 10,000 mg/L are considered sensitive species [4]. It is difficult to say if *P. promelas* would fall into this category given the range of concentrations and durations tested. However, one test organism died in each of the 1000 mg/L MN exposures for each duration tested. This suggests that the 24 hr LC_{10} for MN might be below the 10,000 mg/L criteria. On the other hand, the LC and KN exposures had no mortality in any concentration at any time point.

This trend in mortality caused by MN exposure may be linked to WBNa concentrations. Some researchers have suggested that a 30% loss of sodium from the plasma would be associated with mortality in freshwater fish, but this value is debated among investigators [51-52]. By only using plasma sodium concentrations, sodium ions available in other parts of the body, such as tissues, are ignored. Therefore, WBNa loss might be a greater indicator of organism health due to ion storage outside the bloodstream. Rainbow trout and yellow perch exposed to elevated copper concentrations showed threshold toxicity at 30-40% WBNa loss and a 60% loss caused some mortality [53]. If occasional mortality can be explained by these reported decreases in WBNa, then the deaths seen in the MN should be expected as WBNa concentrations reached 50% of the control concentrations in the 1000 mg/L exposures. This fact stresses the importance of clay source when evaluating suspended solids toxicity.

CONCLUSIONS

This study assessed lethal and sublethal responses of *D. magna* and sublethal responses of *P. promelas* to acute suspended clay-sized particle exposures of varying particle sources. Suspended particles caused significant mortality to *D. magna* at low concentrations; with LC_{50} values ranging from 5 mg/L to 74 mg/L, depending on particle source. Growth and reproduction data showed similar trends in source effects, and although all three particle types caused a reduction in growth, only the lost creek particles caused a significant reduction in reproduction. These results demonstrate that *D. magna* is sensitive to suspended clay-sized particle concentrations that are very common in the natural environment. These concentrations induce effects that may influence population dynamics in aquatic systems. Source and type of clay-sized particles had an effect on D. magna response indicating that bioassays with defined particles may not be predictive of effects in surface waters.

P. promelas showed some sensitivity to the suspended particles, and this sensitivity varied by source. Lost Creek and kaolinite exposures did not cause significant changes in any of the endpoints over any of the concentrations and durations tested. A duration effect from montmorillonite exposure was seen in whole-body sodium concentrations and Na^+/K^+-ATPase activity. Montmorillonite concentration also significantly affected whole-body sodium concentrations. Again, due to the variability in effects between particle sources, it would be unlikely that any defined particles could be used to successfully duplicate effects from natural particles.

This study demonstrated the potential effects of low concentrations of suspended clay-sized particles on fish and invertebrates. Suspended solids concentrations much lower than those measured during storm events caused significant stress to *D. magna* and *P. promelas*. Upon removal from sublethal exposures, *D. magna* appeared to recover rapidly. While sublethal stress caused quantitative changes in biochemical markers in *P. promelas*. The precise method by which suspended solids cause effects in fish is still unclear. Better understanding of how these variables interact will lead to an increased ability to protect organisms from suspended solids induced stress.

FUTURE WORK

Although this research provides insight into how suspended solids affect aquatic organisms, there are several limitations that prevent a full analysis. Although pure clay deposits do exist in the natural environment, this research has shown that modeling organism interaction with suspended solids based on results from pure clay particles is not realistic. Sediment particles in the environment often have adsorbed or absorbed contaminants that may affect organism response to suspended solids. The natural clay-sized particles used in this research were not screened for the presence of common contaminants such as metals or organic pesticides. Therefore, it is possible any or all of the LC results for both organisms could be skewed.

The reason why montmorillonite was more toxic than the other two clay-sized particles remains unexplained. While swelling of the particles post test initiation is possible, this theory needs to be investigated. It is also possible that montmorillonite's greater ion exchange capacity played some role in toxicity. The XRD analysis of the LC particles does also not provide a complete picture of the sediment composition. For example, amorphous solids do not appear in an XRD analysis. X-ray diffraction is meant to provide an estimate only and a full scale analysis would be needed to completely characterize a given sediment source.

Finally, as with all suspended solids testing, there is a large amount of variability in nominal and actual test concentrations of the suspended particles. Adhesion and cohesion play a large role in altering test concentrations, as well as the organisms themselves. Fish will ingest particles and depending on the setup of the test and the number of organisms involved, this may significantly affect circulating suspended solids concentrations. Currently there are no standards methods regarding suspended solids toxicity testing and many researchers have developed their own systems. Most rely on pumps or air stones to circulate test media, but these methods, as noted in this study, do no provide a high degree of control over actual test concentrations. Further work into efficient, precise test methods still needs to be conducted and most testing systems in use today have significant room for improvement.

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